REMARKS

Applicant has amended the claims to more particularly define the invention taking into consideration the outstanding Official Action.

Claims 1-49 have been cancelled from the application and new claims 50-72 have been added. Claims 50-71 correspond to claims 28-49 but have been rewritten as new claims to overcome the outstanding rejections and are fully supported by the specification, including the claims, as originally filed.

The Examiner has maintained a rejection relating to the citation of references. Applicants believe that all relevant references were included in the two IDS's submitted previously on this case, and most particularly with the response of 23 August 2002. It is therefore believed that no further comments are needed with respect thereto.

The rejection of claims 28-49 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention has been carefully considered but is most respectfully traversed.

By way of background to the invention, it is important to appreciate that several distinct relevant species exist in a biological sample and many of these have confusingly similar names. Without bearing in mind the differences between these species at all stages it is impossible to follow and understand the method of the invention. As would be appreciated by one of ordinary skill in the art to which the invention pertains, the application relates to the cobalt containing vitamin "cobalamin" or vitamin B_{12}. This consists of a corrin ring with a complexed cobalt ion and has a molecular weight of around 1350 amu. In contrast "transcobalamin" is a term applied to a group of proteins which bind to cobalamin but are totally different in size and structure to cobalamin itself. "Transcobalamin II" (TCII) is the protein of key interest in the present case and is now believed to be the protein responsible for transfer of cobalamin into cells from the blood.

The two other transcobalamin proteins (TC-I and TC-III) are collectively known as Haptocorrin (HC) and are also present in the blood. In all relevant protein species the prefix "apo-" indicates a protein not bound to cobalamin and the prefix "holo-" indicates the cobalamin bound species. There is much more holo-HC present in the blood than holo-TCII and most of the TCII protein is present as apo-TCII (i.e. without cobalamin bound). Holo-TCII therefore represents a minor portion of the total cobalamin and a minor portion of the total TCII in a biological sample.

Attention is drawn to the Figure submitted previously in this case and designated Figure 1, copy attached. This shows the steps of one embodiment of the invention. The key to the presently claimed invention is the use of an immobilised ligand which binds the apo- species (i.e. the cobalamin free proteins) but does not bind the holo-species. It is evident that a ligand analogous to cobalamin will bind to apo-species because the cobalamin binding site is empty but will not bind to holo-species where this site is already occupied.

In the embodiment shown in the figure, the apo-species bind an immobilised cobalamin ligand and are then removed. Subsequently a TCII binder is added, which binds TCII and allows this to be separated from HC. The bound TCII is then transferred to the final tube. It is evident that the concentration of holo-TCII in the final tube is greater than that in the first tube because the same amount of holo-TCII is present in a smaller volume. It does not matter how much was present originally. The ratio of the first and last concentrations are known providing the ratio of the first and last volumes are known.

Although in the embodiment shown in the Figure the apo-TCII and apo-HC are removed, the assay will work without their removal. This is because the TCII binder cannot bind TCII which is attached to the immobilised cobalamin ligand. Providing the holo-TCII fraction bound by the TCII specific ligand can be selectively removed it does not matter whether the apo-species were physically removed or simply rendered

inactive. The immobilised cobalamin analogue can thus work in one of two ways; either it allows the apo-species to be removed or it prevents them from binding to the TCII specific ligand. This was described originally in the last paragraph of page 16 of the application as filed, which reads;

> *In a preferred embodiment, in the preliminary separation step, the binding of apo TCII and apo HC to cobalamin, analogues or fragments thereof takes place at a site or in such a manner which inhibits subsequent recognition and binding of the immobilised cobalamin bound TCII by the non-immobilised ligand or binding partner for TCII. In this embodiment there is no need to isolate the holo-TCII and holo-HC from the bound apo forms before performing the assay of the invention. In this embodiment, the site against which the non-immobilised binding partner or ligand is directed is very important and should be an epitope on TCII which becomes masked or shielded or otherwise unavailable for binding when the apo TCII and apo-HC becomes immobilised on the cobalamin, analogue or fragment thereof.*

The Examiner objects that "transcobalamin II bound cobalamin" and "holo-transcobalamin II" are "different compounds". As the Examiner will appreciate, and as discussed above, this is not the case since cobalamin bound to TCII is, by definition, holo-TCII. In order to avoid any possible confusion however, the term "holo-transcobalamin II" is now used throughout the amended claims.

In this section, the Examiner states that certain claims are "not clear as to what the immobilised cobalamin will bind". In view of the above discussion, however, it is evident that a worker of ordinary skill in the art to which the invention pertains will be quite clear on this point. Since apo-forms of HC and TCII have, by definition, an empty cobalamin binding site they will bind to immobilised cobalamin. The corresponding holo-species have a cobalamin molecule already occupying this site and so will be incapable of this binding. This is illustrated in the first two steps of Figure 1 previously submitted.

It is respectfully submitted that no worker of ordinary skill in the field would be unclear on this point. Accordingly, it is most respectfully requested that this aspect of the rejection be withdrawn.

Applicants believe that the Examiner is taking the term "analogue or fragment thereof" out of context in this section. In fact, the claim specifies "an analogue or fragment thereof which selectively binds the apo forms of TCII and haptocorrin..". This definition clearly defines the nature of the "analogues" which may act as binders in this claim. In order to clarify this point further, however, claims 28 and 49 now new clams 50 and 71 have been rewritten to read "an analogue or fragment thereof which selectively binds the apo forms of TCII and haptocorrin over the holo-forms thereof..". Applicants believe that this addresses objection C and also further clarifies the situation with regard to point B and most respectfully requests that this aspect of the rejection be withdrawn.

Sections D and E both relate to the question of which species are present at which stage of the assay. It is important to note that it is not essential to the invention that the apo-species be removed following the pre-binding with the immobilised cobalamin. It is only essential that they do not react further. This can be achieved either by removal or by an appropriate choice of ligands such that the immobilised cobalamin blocks the binding of the TCII specific ligand. In order to clarify the situation, claim 28 now new claim 50 has been rewritten to read "wherein the binding of said apo-TCII to said immobilised cobalamin or analogue or fragment thereof is followed by removal of said apo-TCII or renders said apo-TCII unable to bind to said specific binding ligand for TCII or holo-TCII". This is based upon the original disclosure in the last paragraph of page 16. Claim 49 now new claim 71 is believed clear in its existing form since a removal step is explicitly recited. Accordingly, it is most respectfully requested that this aspect of the rejection be withdrawn.

As indicated above and as is clearly illustrated in the Figure previously submitted, it is in no way essential to know the starting concentration of holo-TCII in order to achieve a final holo-TCII concentration increased by a known factor. Since concentration is amount per unit volume, all that needs to be known is the relative amount and the relative volume. These are controlled by the experimenter (in the case of the volume) and by the properties of the ligand (in the case of the amount) and can be easily established in any particular case. As the Figure illustrates, carrying out the separation to yield a relative increase in concentration is therefore simply a case of appropriate binding, separation and release steps as would be evident to a skilled worker. Claim 29 which is now new claim 51 is thus believed clear in this respect. Accordingly, it is most respectfully requested that this aspect of the rejection be withdrawn.

The Examiner's proposed changes to claims 31 and 35 have been introduced to the enclosed claim set as new claims 53 and 57. Claim 35 has been divided into rewritten claim 57 and new claim 72 for clarity. Accordingly, it is most respectfully requested that this aspect of the rejection be withdrawn.

The Examiner objects to the section describing the binding characteristics of the ligands on the basis that it does not clearly define what binding takes place. For clarity and since a ligand will necessarily bind any substrate it is capable of binding, claim 35 now claim 57 and new claim 72 have been amended appropriately to indicate that the substrate is in fact bound.

In order to address the points raised in this section, claims 36 and 37 now claims 58 and 59 have been rewritten to read ".. wherein said specific binding ligand binds holo-TCII with an affinity constant of ...". It is believed that these claims are thereby rendered fully clear and therefore compliant with 35 USC 112.

The Examiner appears to have read claim 39 which is now rewritten as new claim 61 as dependent upon claim 38 now claim 60 where in fact is dependent upon claim 28

now claim 50. There appears to be no inconsistency between claims 28 and 39 now claims 50 and 61 and so Applicants would submit that this claim is acceptable as presently rewritten.

The abbreviation "HC" is now specified as "haptocorrin" in new claim 50.

As proposed by the Examiner, claim 43 now claim 65 has been rewritten into Markush format.

In view of the Examiner's comments, the claims have been reviewed and amended in order to be as consistent as possible and clarify any repeated terms. In claim 34 now claim 56, particularly cited by the Examiner, the term "labelled ligand" has been replaced with "labelled cobalamin analogue" since this is less confusing and better defines the role of this agent. Accordingly, it is most respectfully requested that this rejection be withdrawn in view of the above comments and amendments to the claims.

The rejection of claim 31 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement has been carefully considered but is most respectfully traversed.

The Examiner rejects claim 31 now rewritten as new claim 53 on the basis that only antibodies were described in the specification in sufficient detail for a skilled worker to identify a specific binder of the specified type without undue burden. In the enclosed claim set, new claim 53 has duly been limited to antibodies and antibody fragments as proposed by the Examiner. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The Examiner raises a provisional double patenting rejection in view of US patent Application No. 09/417,226. This application has in fact now lapsed but is continued as 10/897,443. Since the claim set of this application is far from certain Applicants are reluctant at this point to file a terminal disclaimer until there is an indication of allowable subject matter. Accordingly, it is most respectfully requested that this rejection be held in abeyance until there is an indication of allowable subject matter.

The obviousness rejections under 35 USC 103 have been considered. The rejections rely on the teachings of the primary reference of Herbert in combination with certain other documents. As background to this section, it is important to appreciate how the method of Herbert is carried out. Specifically, the method of Herbert works by either crudely separating TCII from HC and then releasing the cobalamin from one fraction or by selectively releasing cobalamin from one species. The released cobalamin is then measured. In any case it is necessary to denature one or both protein components in order to release the cobalamin and obviously this must be a permanent denaturation or the protein would bind to the cobalamin again. The cobalamin may be measured by a "competition assay" in which a labelled cobalamin competes with the released cobalamin for binding to a suitable ligand (such as intrinsic factor). At this stage the labelled cobalamin cannot bind to TCII or HC because these proteins have either been removed or denatured to prevent them re-binding the released cobalamin.

The rejection of claims 28-35, 43, 44 and 49 under 35 U.S.C. 103(a) as being obvious over Herbert in view of Maggio has been carefully considered but is most respectfully traversed.

In section I, the Examiner rejects several claims over Herbert (US 4680273) when combined with Maggio (Immunoenzyme Technique I, 1980, 186-187). It is the Examiner's view is that adding labelled cobalamin to a sample as described in Herbert is, in essence, the pre-binding step described in the presently claimed invention with the exception that Herbert does not use an immobilised cobalamin. In view of the above, however, it is evident that since Herbert is conducting a competition assay for cobalamin from holo-TCII, the TCII must have been denatured to release this cobalamin. The Examiner's assumption, therefore that "the use of cobalamin would necessitate the same binding characteristics" is evidently incorrect because the TCII would be in no state to bind anything as would be appreciated by one of ordinary skill in the art. It is

clear, therefore that all of the obviousness rejections raised in sections I-IV are moot because no document provides the essential apo-protein pre-binding step which is the key feature of the present claims.

It is also noticeable that the Examiner has imported certain portions of this section from previous Office Actions created by Examiner Hines on the copending case. The existence of this reproduction was evident because these sections include obvious misunderstandings of the technology and terminology to an extent that Examiner Cook has not previously exhibited. Applicants most respectfully request that the Examiner readopt her previous positive approach to understanding the present invention and underlying technology and that she review the objections raised by other Examiners in this light before reproducing them. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 40-42 under 35 U.S.C. 103(a) as being unpatentable over Herbert in view of Maggio and in further view of Hoyle et al. has been carefully considered but is most respectfully traversed.

The Examiner simply expands the rejection of the previous section to cover claims 40-42 by reference to Hoyle. As discussed above, Herbert fails to provide any teaching towards an apo-protein pre-binding step because the protein is denatured before contact with any ligands which could bind in the cobalamin binding site. Hoyle makes no teaching relevant to this essential step and thus cannot render the claims obvious in combination with Herbert. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 45-48 under 35 U.S.C. 103(a) as being unpatentable over Herbert in view of Maggio and in further view of Houts has been carefully considered but is most respectfully traversed.

In this section the Examiner again extends the obviousness rejections rejections to cover the remaining claims by incorporation of Houts into the analysis. Houts,

however, provides no teaching relating to the essential point of apo-prebinding and so cannot render the present claims obvious.

One point which Applicants would like to emphasise is that the line "but also cobalamin analogues including TCII, R proteins and intrinsic factor" indicates a lack of appreciation of the different species present in the sample. Cobalamin is a small organic molecule with a bound cobalt which TCII, R-proteins and intrinsic factor are proteins. To use the commonly adopted "lock and key" analogy for enzymes, cobalamin analogues are alternative "keys" in that they fit the same "lock" as cobalamin (i.e. they bind to TCII). The R-proteins and intrinsic factor are alternative "locks" to TCII in that they also accept the cobalamin "key". A technical definition of the term "analogue" may be taken as "any compound chemically related to but not identical with another" (Henderson's Dictionary of Biological Terms 10th ed., 1989, Longman Scientific & Technical, Longman Group UK Ltd, London, UK). Cobalamin does not contain peptide bonds and therefore does not share the chemical structure of proteins and peptides. It was previously believed that the Examiner was aware of these fundamental points and it is noticeable that this section of the Office Action is reproduced from an Action written by Examiner Hines on the copending case in October 1999. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 36-37 under 35 U.S.C. 103(a) as being unpatentable over Herbert in view of Maggio and in further view of Hoyle et al. has been carefully considered but is most respectfully traversed.

Finally, the Examiner introduces Hoyle in relation to claims 36-37. Hoyle does not, however, provide the essential feature of an apo-protein pre-binding step in either disclosure or teaching. The present claims are thus believed inventive in view of all of the cited art. Accordingly, it is most respectfully requested that this rejection be withdrawn.

In view of the above comments and further amendments to the claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested. The Examiner is also invited to telephone the undersigned attorney at the telephone number bellow, to discuss any proposal or for the purpose of clarification of any issue so that the prosecution can hopefully be expedited to an early allowance.

Respectfully submitted,

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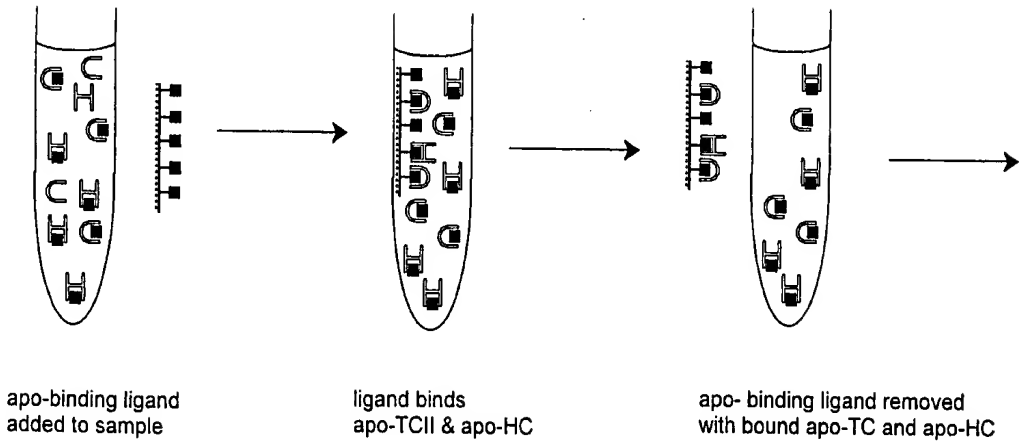

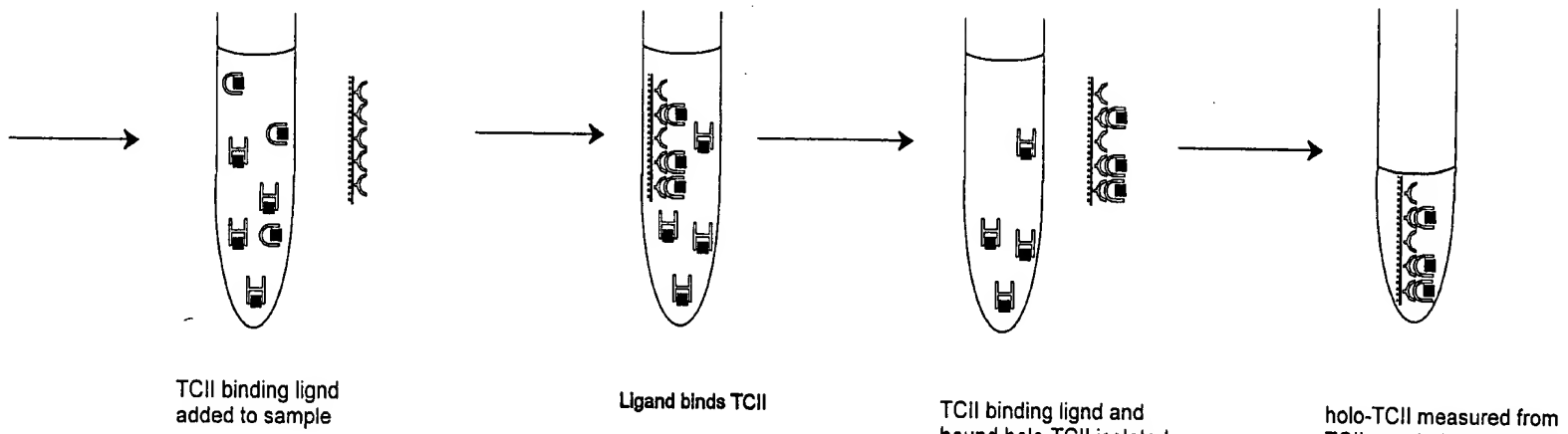

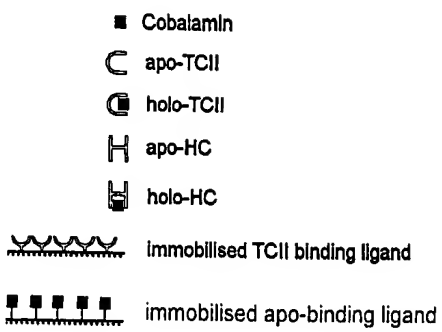

FIG. 1